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(71) Applicant *(for all designated States except US)*: **THE DOW CHEMICAL COMPANY** [US/US]; 2030 Dow Center, Midland, MI 48674 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **BRENNAN, Frank, R.** [GB/GB]; The Dow Chemical Company, Intellectual Property Section - Law Department, 1790 Building, Midland, MI 48674 (US). **HAMILTON, William, D., O.** [GB/GB]; The Dow Chemical Company, Intellectual Property Section - Law Department, 1790 Building, Midland, MI 48674 (US).

(74) Agents: **FISHER, Adrian, John et al.**; Carpmaels & Ransford, 43 Bloomsbury Square, London WC1A 2RA (GB).

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(54) Title: FOOD GRADE SAPONINS AS ORAL ADJUVANTS

(57) Abstract: There is a need for effective oral vaccine adjuvants. Saponin extracts are effective adjuvants but are toxic, so purified saponins have previously been suggested. The invention provides food grade saponins for use as oral adjuvants. This avoids the toxicity problems of the crude extracts and the expense problems of the purified saponin extracts and fractions, whilst simultaneously utilising substances which already have approval for human ingestion.

FOOD GRADE SAPONINS AS ORAL ADJUVANTS

All documents cited herein are incorporated by reference in their entirety.

TECHNICAL FIELD

This invention is in the field of vaccine adjuvants, more particularly adjuvants for use in oral vaccines in mammals (including humans).

BACKGROUND ART

The oral administration of antigens for the purpose of inducing an immune response in some cases requires the addition of an adjuvant. Adjuvants are defined as any substance which is capable of enhancing or augmenting an immune system's response to an antigen or immunogen. Many adjuvants of utility in augmenting humoral immune responses have been described and characterised. However, not all adjuvants which function to enhance immune responses in the circulation are effective in mounting mucosally (including orally) stimulated immune reactions. Among adjuvants which function in systemic applications cholera toxin (CT) and *E.coli* heat-labile toxin (LT) are known to be powerful mucosal adjuvants. Not surprisingly, however, these molecules are unsuitable for oral application to humans due to their toxic nature. A substantial research effort is devoted to producing and characterising mutant variants of these enterotoxins which display attenuated toxicity but which retain oral adjuvanticity [*e.g.* reference 1]. Clinical-grade preparations of mutants of these toxins are not widely available, however, and regulatory approval world wide for their use in orally-mediated healthcare applications is currently awaited.

One antigen which is deemed to be a oral poor immunogen is the surface antigen of hepatitis B virus (HBsAg). To date, only CT has been demonstrated to adjuvant an immune response to this antigen when it is presented orally. Even then, it appears that any adjuvanticity is possible only when preceded by an immune priming event such as a subcutaneous injection of the antigen. Other than the enterotoxins CT and LT, there are very few adjuvants for the augmentation of oral antigens currently known.

Thus there is a need to identify alternative mucosal, especially oral, adjuvants which can be applied in oral/mucosal immunogenic compositions.

One group of substances which has been reported as an oral adjuvant is the saponins – crude *Quillaja* (also known as *Quillaia*) *saponaria* saponin preparations and purified extracts have

both been reported to be effective oral adjuvants [*e.g.* references 2, 3, 4, 5, 6 *etc.*]. Saponins are high-molecular-weight glycosides, consisting of a sugar part linked to a triterpene or steroid aglycone, and occur in over 500 plant genera. The classical definition of saponins is based on surface activity – many saponins have detergent properties, produce foamy suds when mixed with water, and show some haemolytic activity. Consequently, some saponin-containing plants have been employed for hundreds of years as soaps and therefore the names of such plants reflect this: soapwort (*Saponaria officinalis*), soaproot (*Chlorogalum pomeridianum*), soapbark (*Quillaja saponaria*), soapberry (*Sapindus saponaria*), and soapnut (*Sapindus mukurossi*). The most common sources of saponins are the higher plants, but increasing numbers are being found in lower marine animals. So far, they have been found in the marine phylum *Echinodermata* and in species of the classes *Holothuroidea* (sea cucumbers) and *Asteriodea* (starfishes).

Hence, saponins are defined according to their structure. Chemically, saponins are glycosides containing one or more mono- or oligo-saccharide groups linked *via* a glycosidic bond to non-polar aglycone moieties. The aglycone components of saponins are typically steroid or triterpenoid moieties. The aglycone or non-saccharide portion of the saponin molecule is called the genin or sapogenin. Depending on the type of genin present, the saponins can be divided into three main classes: triterpene glycosides, steroid glycosides (*e.g.* medicinal wild yam source) or steroid alkaloid glycosides. The saponins extracted from the bark of *Quillaja* species fall within the triterpene glycoside classification of saponins.

Such saponins are concentrated in extracts made from the bark of the Chilean soap bark tree and comprise a heterogeneous mixture of triterpene glycosides and other molecules present in the native extract.

Crude saponins are generally too toxic for human use, however – *Quillaja saponaria* is, for instance, listed in *Poisonous Plants of California* [7]. It has therefore been perceived as necessary to purify them for use as adjuvants, with an enriched sub-fraction known as QuilA and its further purified QS21 fraction receiving particular attention [*e.g.* reference 8]. Indeed, the assignee of US patent 5,057,540 has stated that, "to achieve a safety profile acceptable for human use", a saponin fraction should show a single predominant peak (≥90% area) in HPLC analysis [9], such as the QS21 fraction.

Preparing saponins to such high purity is inconvenient and expensive, however, and it is an object of the invention to simplify the use of saponins as adjuvants in vaccines.

DISCLOSURE OF THE INVENTION

The invention is based on the surprising discovery that saponins retain acceptable oral toxicity and adjuvanticity profiles without being subjected to the high degree of purification previously thought to be necessary. In particular, "food grade" saponins (FGSs) are effective oral adjuvants. This runs wholly contrary to the advice given, for instance, by the European Centre for the Validation of Alternative Methods in reference 10, where it is specifically stated that, "Saponin preparations intended for use as immunological adjuvants (for example, QuilA or QS-21) are purified to reduce the presence of components which cause adverse local reactions. Food-grade saponin preparations should not be used for immunization schemes."

Thus, the present invention demonstrates for the first time alternative immune compositions which incorporate food grade saponins in conjunction with known orally refractory antigens, gut-pathogen derived epitopes, and epitopes, antigens or immunogens which are capable of initiating an immune response *via* mucosal surfaces.

The present invention relates to the induction of mucosal and systemic immune responses by means of oral administration of immunogenic compositions containing food grade saponins. In particular, it relates to the use of food grade saponin preparations admixed or co-administered with immunogenic compositions to elicit both a mucosal and/or a systemic immune response which is augmented over that detected in the presence of the antigen or immunogen alone. In one aspect the invention discloses the use of food grade saponins to augment the immune response to an antigen when presented orally. This aspect of the invention is particularly pertinent to prophylaxis or immune therapy directed against gastro-intestinal and urino-genital infections or disease agents, as well as any pathogens whose biology necessitates interaction with any mammalian mucosal surface. Hence, the present invention contemplates the prophylaxis or treatment of in principle any mucosally mediated disease or infection in addition to providing a means for eliciting an immune response initiated at the mucosa.

In a further aspect, the invention discloses the use of food grade saponins to boost the immune response specifically to an antigen which has been used to prime the immune system of an individual. This is particularly useful for boosting a primed but critically low level immune reaction to HBsAg. In principle, antigens may be expressed in cell free, unicellular or

multicellular expression systems and purified, enriched or otherwise prepared for oral or mucosal delivery by a variety of processes known and described in the art. More recently, it has been demonstrated [*e.g.* refs 11, 12, 13 *etc.*] that antigens can be expressed in transgenic plants (*e.g.* potato, tomato, spinach, alfalfa, carrot) and minimally processed prior to presentation to the immune systems of animals, in particular mammals including man. Thus, in one embodiment of the present invention hepatitis surface antigen (HBsAg), expressed in transgenic potatoes, is presented with food grade saponin adjuvant. Amongst further embodiments, the present invention applies to other antigens expressible in plant material such as that derived from tubers of potatoes, fruit of tomato plants and so on. Antigens may include virus epitopes such as those found in Norwalk virus coat protein [14], respiratory syncytial virus F protein or HIV pg41 *inter alia*; bacterial epitopes including those derived from fibronectin-binding protein of *Staphylococcus aureus, Staphylococcus epidermis*, or the urease of *Helicobacter pylori* among others; fungal epitopes diagnostic for *Candida albicans* and other pathogenic or infectious fungi; and altered "self" epitopes corresponding to variant endogenous proteins which are frequently associated with solid tumours or metastatic cancers to name but a few. Other viruses with antigens for use with the invention are caliciviruses including small round structured viruses (SRSVs), Norwalk virus, Southampton virus, Snowy Mountain virus and Chiba 104 virus.

In yet another aspect the present invention a means is provided of augmenting an immune response when the amount of available immunogen is limited by technical or supply considerations such as stability, or production levels *in vitro* and so on. In an extension of this aspect of the present invention, a means of raising specific antibodies to low abundance or poorly immunogenic antigens is provided. Such antibodies may find applications as reagents of utility in cell biology, proteomics, affinity purification and diagnostics.

The term "food grade" is widely used by manufacturers to describe saponins (see below) and in the art [*e.g.* ref. 15]. A "food grade" saponin is defined herein as any grade or preparation of saponin which is approved for use in food and beverages under the United States Food and Drug Administration (FDA) regulation 21 CFR 172.510, FEMA GRAS number 2973 and/or the European Commission code E 999. "Food grade" saponins are at a higher purity than untreated bark extracts, but a lower purity than QuilA, the purified HPLC fractions described in reference 8, and analytical grade saponins. The adjuvanticity of *Quillaja* saponins had been assumed to reside in the partially defined fractions (*e.g.* QuilA and QS21) or in essentially

untreated extracts and, in the absence of evidence to the contrary, the adjuvanticity had evidently been seen a property only of the purified fractions or unprocessed crude extract.

The use of "food grade" saponins offers several advantages as adjuvants. In comparison with QuilA, QS21 *etc.* the adjuvants can be advantageously prepared much more cheaply. Indeed, this grade of saponin is commercially available as a food additive in a form suitable for use according to the present invention, from suppliers such as: Natural Responses S.A., Chile (Product Codes QL 1000 and QP 1000 *inter alia)*; Berghausen Corporation Inc., 4524 Este Avenue, Cincinnati, Ohio 45232, USA (Foamworx, Baker's Preferred and Technical Grade Saponins); Garuda International, Inc., PO Box 5155, Santa Cruz, California 95063, USA (Product Code QUEXT100); Frutarom Meer Corporation, 9500 Railroad Avenue, North Bergen, New Jersey 07047-1206, USA (Food grade *Quillaja* extract batch No. 2754810); Schweizerhall, Piscataway, New Jersey, USA (Saponin Food Grade 100%); Advance Scientific, Fort Lauderdale, Florida, USA (SAP230 SAPONIN, Powder, Food Grade); Voigt Global, Topeka, Kansas, USA (Saponin Powder (*Quillaia* Extract) 8047-15-2, Food Grade).

In comparison with crude or unprocessed bark extracts, which are unlikely to be accepted for introduction into the human food chain (*e.g.* "Harmful if swallowed" warning in reference 16), the adjuvants advantageously conform to FDA and EC regulatory approvals for human consumption. Clearly, "food grade" saponins are considered safe for human ingestion. Indeed, food grade *Quillaja* extracts are used at concentrations of up to 200mg/kg in soft drinks and are used in a range of food products as emulsification and foaming agents.

Whilst food grade saponins have previously been disclosed for ingestion, however, it had not been appreciated that they would function as oral immunological adjuvants. In particular, even though the saponins have been present in foodstuffs, they have not been reported to promote adverse immune reactions to the constituent components of the drinks (*e.g.* beers) or other ingestibles in which they are present. This would have suggested, contrary to the present invention, that they do not possess adjuvant-like characteristics. Moreover, there is abundant evidence that saponins are poorly absorbed in the gut and are hydrolysed to their constituent sapogenins and sugar moieties [17]. Now, according to the present invention, it has surprisingly transpired that food grade saponins serve to augment the responses to antigens when presented orally to mammals in regimens typified by the examples presented below.

To address issues of toxicity, saponins (*e.g.* QuilA) are typically incorporated into structures known as ISCOMs [*e.g.* references 18 & 19]. It is a further advantage of the invention that ISCOMs need not be used. Indeed, it is specifically preferred that the saponins are not used in the form of ISCOMs.

Thus the invention avoids the toxicity problems of the crude extracts and the expense problems of the purified saponin extracts and fractions, whilst simultaneously utilising substances which already have approval for human ingestion.

Preferred FGSs for use according to the invention are the Frutarom and Berghausen products.

A preferred antigen for use according to the invention is HBsAg, in any of its various forms (*e.g.* S, preS1, preS2, the 2F10 peptide *etc.*). Other preferred antigens are CVPs carrying immunogenic epitopes. Antigens of the invention may be presented on a carrier macromolecule (*e.g.* KLH).

Vaccines of the invention will typically comprise carriers in addition to the antigen and the saponin adjuvant, and may contain diluents, such as water, saline, glycerol *etc.*

The vaccines will usually comprise an immunologically effective amount of antigen *i.e.* that amount which, when administered to an individual, either in a single dose or as part of a series, is effective for treatment or prophylaxis. This amount may vary depending upon the health and physical condition of the individual to be treated, the taxonomic group of individual to be treated (*eg.* human, non-human primate, primate, *etc.*), the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. The amount will fall in a relatively broad range that can be determined through simple trials. Appropriate dosages of saponin adjuvant can be determined similarly.

Definitions

Adjuvant – any composition, physical or chemical entity whose presence with an immunogenic or antigenic determinant results in an increased level of immune reaction when compared with the immune reaction realised with the immunogen or antigen alone.

Antigen – a macromolecule or other physical entity which upon introduction into animals, especially mammals (including humans) is capable of stimulating the production of immunoglobulins which interact with and bind specifically to the said antigen. For clarity, the

word "antigen" as used herein is intended to include epitopes, antigenic determinants, any fusion protein which includes an antigen per se or an antigenic determinant thereof.

Antigenic determinant - physical structure which determines specificity of an immune reaction

Chimaeric virus particle – any plant virus genetically modified to express epitopes or peptides in its coat protein [*e.g.* references 20, 21, 22, 23 *etc.*]

Immunogen – an antigen as defined above which is capable of activating an immune response *in vivo* which may include eliciting immunoglobulins specific for antigenic determinants, activation of complement pathways and activation of T cells *inter alia* effective in reducing or ablating the deleterious effects of the presence of the antigen in the system of the host.

Quillaja – means *Quillaja saponaria* Mol., also known as *Quillaia, Quillaia saponaria* Mol. and the Chilean Soap Bark Tree.

Secretory immune response – means the production and formation of secretory immunoglobulins in secretions which bathe the mucosal surfaces of animals (including humans) or in secretions from secretory glands. A secretory immune response may be referred to as a mucosal immune response.

MODES FOR CARRYING OUT THE INVENTION

The present invention is further described below with reference to the following examples. Unless otherwise stated, in all the examples positive controls are provided through the use of cholera toxin as the oral adjuvant. This control is included along with a negative control in each example (no adjuvant added) to confirm that the level of immune reaction to a particular test antigen or immunogen *per se* can be augmented.

Example 1. – use of food grade saponins in immunogenic compositions to boost animals previously primed with HBsAg vaccines

Female BALB/c mice (H-2^d), aged 6-8 weeks, were immunized subcutaneously with 0.5μg HB-VAXII40™ (Recombivax™, Pasteur Merieux) or 1μg Engerix-B™ (SmithKline Beecham), both of which are adjuvanted with alum. On day 51 the mice were bled and, on days 55, 57 and 59, fed 5g HB114-16 cubes (derived from potatoes expressing HBsAg). The potato material was spiked with either CT (Sigma; 20μg CT *per* 5g potato) as a positive control or Frutarom food grade liquid *Quillaja* extract (10mg saponin *per* 5g potato). Sera

were collected from the mice before feeding (day 51) and after feeding on days 63, 72 and 79. Sera were assayed using a sandwich ELISA kit (Biokit, Barcelona, Spain) and the serum anti-HBsAg titres (mIU/ml) were determined according the manufacturers instructions:

Priming Ag	Booster adjuvant	Mouse	Day 51	Day 63	Day 72	Day 79
HB-VAX II	CT	D1	0	0	0	0
		D2	0	0	0	0
		D3	0	0	0	0
		D4	0	0	0	0
		D5	1446	0	0	0
		D6	0	0	0	0
		D7	0	0	0	0
		D8	>2000	0	0	0
		D9	0	0	0	0
		D10	0	0	0	0
	FGS	E1	228	1528	3820	3894
		E2	1018	>8000	>18000	>18000
		E3	0	0	0	1278
		E4	0	0	0	120
		E5	0	2528	3708	4290
		E6	0	0	0	208
		E7	0	>8000	808	810
		E8	0	318	4634	4056
		E9	1082	2303	>18000	>24000
		E10	0	0	0	2466
Engerix B	CT	F1	0	578	1000	659
		F2	159	1010	1512	1522
		F3	>4000	5818	>16000	>18000
		F4	1014	>8000	>16000	.24000
		F5	5894	>8000	>16000	>18000
		F6	812	2206	4704	>12000
		F7	0	0	0	542
		F8	0	86	0	233
	FGS	G1	2424	>2000	3232	2898
		G2	2367	>8000	>16000	>18000
		G3	76	1820	7010	6899
		G4	532	3094	3464	3912
		G5	0	174	340	804
		G6	0	226	132	238
		G7	>8000	>8000	>16000	>24000
		G8	210	1325	3118	6950

In animals primed with HB-VAXII40™, none of the mice responded to the CT-adjuvanted HBsAg booster (no increased titres of HBsAg-specific mIUs). In contrast, all (10/10) of the

mice boosted with FGS-adjuvanted HBsAg responded with significant increases in serum titres, although the individual responses between mice varied considerably.

Interestingly, all mice primed with Engerix-B show a boost in titre following feeding of HBsAg-expressing potatoes together with CT or FGS, suggesting differences in levels or kinetics of priming achievable with these two commercially available vaccines.

Example 2. – further study with HBsAg

Example 1 was repeated, except that: (i) only the Pasteur vaccine was used for priming; (ii) the FGS used was from Berghausen. Representative end titres for 16 mice are shown:

Animal	CT-adjuvanted booster			FGS-adjuvanted booster		
	Pre-boost	*Day 60*	*Day 70*	*Pre-boost*	*Day 60*	*Day 70*
1	0	200	**400**	25600	12800	25600
2	400	400	**800**	1600	800	1600
3	800	400	**1600**	800	1600	**3200**
4	400	400	**800**	3200	3200	**12800**
5	200	0	0	12800	6400	**25600**
6	0	0	**200**	25600	12800	**102400**
7	0	0	0	3200	800	**6400**
8	3200	3200	**6400**	3200	3200	**25600**

Titres which appear to indicate a booster effect are indicated in bold type. The FGS is clearly an effective adjuvant for boosting using HBsAg.

Example 3. – toxicity and immunogenicity evaluation with influenza haemagglutinin

Influenza haemagglutinin (HA) is more commonly encountered at mucosal surfaces than HBsAg and was used as a test antigen in mice. To assess toxicity, various concentrations of Frutarom FGS (500mg/ml) were evaluated. Vaccines were administered on day 0 and day 7, with serum anti-HA titres determined after 28 days:

Mouse	HA dose	FGS dose	End point titre
A	20µg	10mg	0
B	20µg	4mg	1600
C	20µg	2mg	0
D	20µg	1mg	400
E	20µg	0.5mg	0

No toxicity was observed in any animals. The lack of anti-HA response in mice A, C and E seems to result from the short immunisation schedule (days 0 & 7). In other experiments, where a day 14 dose was also given, anti-HA titres reached as high as 204800.

Example 4. – comparative study of adjuvants using HA antigen

The following adjuvants were tested using HA:

Adjuvant	Source	Dose
CT	Sigma	20μg
LT-R72 [24]	Chiron Corporation	20μg
QS-21	Aquila Biotech	50μg
FGS	Frutarom	1mg & 10mg
FGS	Garuda	1mg & 10mg
Purified *Quillaja* extract (non-FGS)	K Dalsgaard	10mg

Five female BALB/c mice, aged 6-8 weeks, were immunised in each group by oral gavage on days 0, 2 and 28. Anti-HA titres were assessed by ELISA after days 42 and 56. The best end-point titres were seen in the group receiving Frutarom FGS (1mg). Again, no toxicity was observed using the FGS.

Example 5. – the use of food grade saponins to prime an immune response to a viral epitope (Norwalk virus capsid protein [NVCP])

CD1 mice are fasted for at least 10 hours during daylight before consuming minimally processed (peeled and cubed) tuber material derived from transgenic potato plants expressing NVCP fed to test animals overnight. The mice in three test groups (each group containing 5 mice) are bled on day 0 prior to subsequent feeding on days 1, 2, 11 and 28 with variously 5g tuber transgenic material *per* mouse. As a positive control, the tuber material in Group 1 is spiked with 20μg of CT *per* 5g dose; the remaining tuber material is spiked with 10mg FGS *per* 5g dose (Group 2) or left untreated (Control Group 3). The mice from all three groups are bled on days 12, 21 and 42 post-feeding and the level of anti-NVCP immunoglobulins determined by an ELISA essay. Those mice fed with potato cubes with added CT or FGS show an increase in immunoglobulins specific for NVCP over that seen for the mice that are fed transgenic potato material expressing NVCP alone.

Example 6. – the use of food grade saponins orally to prime an immune response to respiratory syncytial virus protein F

Fruit from transgenic tomatoes expressing the human respiratory syncytial virus (RSV) Fusion Protein is treated with respectively 20μg cholera toxin *per* 5g tomato or 10mg food grade saponin *per* 5g tomato. A third sample of transgenic fruit material is left untreated. On day 0 pre-immune sera are collected from all test mice in the three groups. Three test groups are fed between 5 and 7g of tomato fruit, corresponding to approximately 190μg RSV protein F with variously no adjuvant (Group 1) CT (Group2) or FGS (Group3) on days 1.2 12 and 28. On days 12, 21 and 42 after the final feed tail bleeds are carried out on all the test animals in the three groups and the levels of immunoglobulins specific for protein F of human RSV are determined by ELISA. The animals in Groups 2 and 3 produce higher mean titres of immunoglobulins specific for RSV protein F than those in Group 1 (fed on tomato material with no added oral adjuvant).

Example 7. – use of food grade saponins to augment an immune response to a mucin epitope conjugated to a protein carrier, keyhole limpet haemocyanin (KLH).

A KLH conjugate containing a synthetic polypeptide corresponding to an epitope on the variant human polymorphic epithelial mucin (PEMIp) protein, mucin, encompassing amino acid residues GVTSAPDTRPAPGSTA is re-suspended to final concentration of 1 mg/ml in phosphate buffered saline (PBS) following conjugation according to standard procedures. Pre-immune sera are collected on day 0. The resultant suspension is administered to three groups of test mice (BALB/c) by gastric intubation ("gavage") in the presence of respectively, 20μg CT or 10mg FGS plus KLH:mucin conjugate or without any adjuvant on days 1, 2, 12 and 28. Blood is collected from the test animals on days 12, 21 and 42 post-gavage and the levels of immunoglobulins specific for the mucin peptide epitope realised in the presence of adjuvant compared to the immune reaction triggered in their absence is determined by ELISA. In those test animals exposed to the mucin peptide in the presence of either CT or FGS, the mean titres of immunoglobulins specific for the mucin peptide are higher than those seen in animals challenged with KLH:mucin only.

Example 8. – use of food grade saponins in dogs in immunogenic compositions containing chimaeric plant viruses (cowpea mosaic virus, CPMV) expressing a parvovirus epitope

Two groups of specific-pathogen-free [(SPF) specified free from a series of pathogens including canine parvovirus] beagle dogs (six dogs in each group), age 9-12 weeks, are fed

CPMV-PARVO1 containing a 17-amino acid peptide (DGAVQPDGGQPAVRNER) corresponding to residues 3 to 19 from VP2 of canine parvovirus. This epitope is also known as the 3L17 peptide. Group 1 dogs receive the chimaeric virus only: Groups 2 dogs receive the chimaeric virus with 10mg FGS *per* dose. A dose contains either 7.5 mg of CPMV-PARV01 (containing approximately 150 µg of 3L17 peptide) mixed with FGS diluted in PBS or diluted in PBS without FGS to a final volume of 1.5 ml. Blood is collected prior to application of the orally administered chimaeric virus-containing immunogenic complex. The dogs receive doses on days 1, 2 and 7. Blood is collected on days 28, 38. and 56 and analysed by ELISA for the levels of VP2/3L17 specific immunoglobulins. Those dogs which receive the 3L17 presenting viruses in conjunction with FGS show a higher level of VP2/3L17-specific immunoglobulins than those which receive the antigen alone.

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

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[19] Rimmelzwaan & Osterhaus – Chapter 23 in *Vaccine Design: the subunit and adjuvant approach* (eds. Powell & Newman, ISBN 0-306-44867-X).

[20] EP-B-0580635; see also US patent 5,874,087.

[21] WO96/02649

[22] Brennan *et al.* (1999) *J. Virol.* 73:930-938.

[23] Dalsgaard *et al.* (1997) *Nature Biotech.* 15:248-252.

[24] WO98/18928

CLAIMS

1. An oral vaccine composition, comprising: (a) an antigen, and (b) a food grade saponin.

2. The composition of claim 1, wherein the saponin is derived from *Quillaja* extracts.

3. The composition of claim 1 or claim 2, wherein the saponin is approved for food and beverage use under:
   (a) European Commission Code E 999, and/or
   (b) US FDA 21CFR 172.510 (FEMA GRAS number 2973)

4. The composition of any preceding claim, wherein the antigen is an immunogen.

5. The composition of any preceding claim, wherein the antigen is taken from the group containing epitopes derived from polypeptides or parts thereof expressed by pathogens borne by or representative of mycobacteria, viruses, bacteria, fungi, insects, plants or animals.

6. The composition of any preceding claim, wherein the antigen is taken from the group containing animal tumour-associated, metastasis-associated or cancer epitopes.

7. The composition of any preceding claim, wherein the antigen is in the form of (i) a chimaeric virus particle or (ii) transgenic plant cells or tissue.

8. The composition of claim 7, wherein the antigen is hepatitis B surface antigen expressed by a transgenic plant.

9. The composition of any preceding claim, wherein the saponin is Frutarom or Berghausen food grade saponin.

10. The composition of any preceding claim, wherein components (a) and (b) are ad-mixed.

11. A method for enhancing an immune response to a specific antigen, comprising the step of administering an effective dose of the composition of any one of claims 1 to 10 to a mammal, thereby raising the levels of immunoglobulins specific for said antigen.

12. The method of claim 10, wherein a secretory immune response is induced.

13. The method of claim 11, wherein the immune response is a booster response.

14. The method of claim 13, wherein the priming response was initiated by a non-oral delivery route.

15. A composition comprising (a) an antigen and (b) food grade saponin, for use as a vaccine.

16. Food grade saponin for use as a mucosal vaccine adjuvant.

17. Saponin according to claim 16, wherein the mucosal adjuvant is an oral adjuvant.

18. The use of food grade saponin in the manufacture of an vaccine.

19. The use of claim 18, wherein the vaccine is an oral vaccine.

20. The use of claim 18 or claim 19, wherein the saponin is derived from *Quillaja* extracts.

21. The use of any one of claims 18 to 20, wherein the vaccine is for prophylactic or therapeutic use.

22. A process for manufacturing a vaccine, comprising admixing (a) an antigen and (b) food grade saponin.

23. The composition of claims 1 to 10, adapted for oral administration.